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Due to the localization of matrix metalloproteinases (MMPs) in numerous human breast tumors, these proteins have been hypothesized to be involved in development and progression of breast cancer. We propose that matrilysin, an epithelial specific MMP, is partly responsible for the remodelling of the extracellular matrix during mammary development and tumorigenesis. To test our hypothesis, transgenic mice have been generated that overexpress matrilysin in mammary epithelial cells. Preliminary data indicates that overexpression of matrilysin induces excess branching during mammary development. Induction of mammary tumors are currently in progress. These studies will help address whether matrilysin plays a role in mammary tumorigenesis.

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THE ROLE OF MATRILYSIN, A MATRIX METALLOPROTEINASE, IN MAMMARY TUMORIGENESIS

INTRODUCTION:

Cell-matrix interactions are an important aspect to many biological processes. During processes such as mammary growth and neoplasia the extracellular matrix (ECM) is continuously degraded and remodeled. Proteins that degrade the extracellular matrix, such as matrix metalloproteinases (MMPs), clearly play a role in the interactions that occur within the extracellular environment. We have previously hypothesized that matrilysin, an epithelial specific MMP, is partly responsible for remodelling of the ECM during mammary development and tumorigenesis. To test our hypothesis, transgenic mice expressing matrilysin under the control of the mouse mammary tumor virus (MMTV) promoter/enhancer will be evaluated to investigate if overproduction of matrilysin alters mammary development and/or mammary tumorigenesis.

BACKGROUND:

Extensive remodeling of the ECM in addition to structural changes in the mammary gland occur during mammary ductal development, lactation, and involution. Temporal expression of several MMPs including stromelysin-1 (1),72kDa gelatinase A (1,2), stromelysin-3 and TIMP-1 (1). Matrilysin gene expression has also recently been detected in the adult cycling, lactating, involuting (3), and developing (unpublished observations) murine mammary gland. Although matrilysin expression has not yet been localized to a specific region of the mammary gland it has previously been localized to epithelial cells of other various tissues (3,4). If matrilysin is localized to the epithelial cells of the mammary ducts, we can envision a role for matrilysin in both development and differentiation of the mammary gland.

Several cases of human breast adenocarcinomas have recently been examined for matrilysin expression. Matrilysin mRNA was detected in fibroadenomas and invasive ductal carcinomas (5) and by in situ hybridization in the neoplastic epithelial tumor cells of several adenocarcinomas (5,6). Human matrilysin was first identified by low stringency screen of a mixed tumor library with the intent of cloning stromelysin-related genes that may be involved in tumor progression (7). Other investigators have detected matrilysin mRNA in adenocarcinomas of the human prostate (8), rectum (9), and breast (5). Recently, matrilysin gene expression by colon adenocarcinoma cells has also been suggested to contribute to the tumorgenicity and progression of colorectal tumors (10).

Although matrilysin is highly expressed in many human breast neoplasias, only low levels of matrilysin can be detected in normal human breast tissue that surround neoplastic tissue (5). Similar results have also

been observed in murine mammary tumors as well as normal mammary tissue (unpublished observations). Therefore, we propose that the mis-expression of matrilysin in breast adenocarcinoma tumor cells may contribute to the progression, invasion, or metastasis of these cells.

With the onset of tumor development also comes changes in the tissue morphology and cellular differentiation. The penetration of the basement membrane, which is a hallmark of malignant tumor progression, has some similarities to the remodeling process that is observed during mammary gland development. We have previously shown that low levels of matrilysin gene expression can be detected in the developing, lactating, involuting and cycling adult murine mammary gland while increased expression of this MMP is found in metastatic murine mammary tumors (unpublished observations and (3)). Expression of other MMPs have also been associated with the onset and progression of murine mammary tumors. Stromelysin-1 protein and TIMP-1 mRNA were found in the myoepithelial cells surrounding pre-neoplastic lesions in mammary tumors produced by WAP-ras transgenic animals, but absent in the well-differentiated and non-metastatic tumors produced by the WAP-c-myc transgenic animals (11). In a similar model system, mammary tumors that were induced by 7,12-dimethylbenzanthracene (DMBA) treatments in MMTV-stromelysin-1 and MMTV-TGFa transgenic animals expressed endogenous mouse stromelysin-1 in the stromal tissue surrounding non-invasive or focally invasive adenocarcinomas (12, and J. Witty personal communication). Taken together, these studies establish that tissue surrounding induced murine mammary tumors express a metalloproteinase and its inhibitor, suggesting that regulation of this family of enzymes may be important for the prevention of mammary malignancies.

RESULTS:

Establishment of Transgenic Lines

To test the hypothesis that mis-expression of matrilysin expression contributes to altered mammary development and tumor progression in mammary malignancies, we have chosen to generate a transgenic mouse model system. Three separate constructs have been used to develop three different transgenic lines: 1) a native, or wild-type transgene, 2) a constitutively activated transgene, and 3) an inactive matrilysin transgene. The constitutively active construct contains a mutation that results in spontaneous activation of the enzyme, therefore circumventing any dependence on activation by exogenous factors. A comparison of the results from the native and active matrilysin constructs will give an indication of the availability of activators of matrilysin in the mammary environment. The third construct encodes a matrilysin protein that lacks proteolytic activity due to the presence of an inactivating mutation. The use of this mutant will

determine if any observed effect of matrilysin in this model is due to its proteolytic activity.

All matrilysin constructs were generated by inserting a full-length 1.1 kb fragment of each form of the human matrilysin cDNA into the EcoRI site of the MMTV-LTR expression vector (Figure 1A). Mutations in the construct have been made by site-directed mutagenesis using standard oligonucleotide-directed mutagenesis (gifts from Paul Cannon, Syntex Research). The expression vector contains splice sites derived from the rabbit ß-globin gene, and has been previously demonstrated to be an effective expression vector for transgenic mice (13).

The active matrilysin cDNA contains a valine to glycine substitution in the highly conserved sequence PRCGVPDV, which corresponds to amino acids 88-95 (Figure 1B). This sequence is found near the carboxyl terminal end of the pro-domain. Mutations in the rat stromelysin-1 sequence in this same region leads to variants which have a significantly increased tendency to spontaneously generate active stromelysin-1 (14). When a breast cancer cell line was transfected with matrilysin cDNA carrying mutations within this sequence, immunoprecipitated from the conditional medium by a polyclonal antibody against human matrilysin, and run on a SDS polyacrylamide gel, an increased tendency to undergo spontaneous conversion to a lower molecular weight form corresponding to the mature cleaved activated form of matrilysin was also observed (Figure 1C). This data indicates that this mutation in the matrilysin cDNA confers constitutive activation upon the enzyme and gives similar results as the stromelysin-1 mutation (14).

The catalytically inactive matrilysin cDNA contains a glutamic acid to glutamine substitution at amino acid 216 (Figure 1C). Mutation of the two histidines or the glutamic acid in the rat stromelysin-1 sequence VAAHELGHSLGLFHS (amino acids 213-227), leads to variants which are unable to degrade casein, a substrate of stromelysin, and eliminates the capacity to autocleave during activation (14). These results indicate that the substitution at amino acid 216 inhibits the cleavage and full activation of this enzyme. Since these sequences are highly conserved among MMP family members, we hypothesized that a similar mutation in the matrilysin cDNA would produce a catalytically inactive matrilysin protein. To test this, the inactive mutant and the native construct were transfected into a breast cancer cell line and immunoprecipitated as previously described. When activated by the organic mercuride 4-aminophenyl-mercuric acetate (APMA), which is a known MMP activator (15), the native protein construct was cleaved and converted to the mature form (Figure 1C). In contrast, the inactive mutant protein remained in the higher molecular weight proenzyme form, indicating that this mutation prevents the enzyme from becoming cleaved and therefore fully activated (Figure 1C).

In all lines, the human matrilysin cDNAs are expressed using the murine mammary tumor virus long terminal repeat (MMTV-LTR) as the promoter. The MMTV-LTR has been shown to be expressed in the epithelial cells of virgin and lactating mammary glands, salivary glands, lungs, kidneys,

testes, and lymphoid cells of the spleen and thymus (16) Several studies have shown that the long terminal repeat of this virus can direct the expression of reporter genes to the same tissues in transgenic mice (1,13). In addition, inducability of the MMTV promoter has been previously demonstrated using dexamethasone, a synthetic glucocorticoid (17). We have also shown that our MMTV-matrilysin constructs can be induced in vitro with dexamethasone (Figure 1C).

Plasmids containing each transgene were purified and microinjected into FVB fertilized murine eggs. Transgenic mice were then identified by Southern blot analysis of tail DNA using the full length human matrilysin cDNA, and founder mice harboring the transgene were mated to establish transgenic lines. At least two lines per construct have been or will be established to control for insertional variation (Figure 1D). The resulting transgenic lines will be referred to hereinafter as MMTV-ActMAT (MMTV-active-matrilysin), MMTV-InMAT (MMTV-inactive-matrilysin), and MMTV-NaMAT (MMTV-native-matrilysin). The MMTV-ActMAT lines are approximately 1-5 copies, the MMTV-InMAT 10-30 copies, and MMTV-NaMAT 15-20 copies.

Initial Characterization of Transgenic Animals

In the attempt to analyze the transgene expression, mammary glands were removed from female transgenic and nontransgenic littermates at various times during mammary development (6-17 weeks). The left thoracic and inguinal glands were removed, frozen on dry ice, and poly A(+) RNA isolated with oligo dT cellulose from total RNA obtained by extraction with guanidinium isothiocyanate for northern blot analysis. In addition, right thoracic glands were processed for whole mount staining, while right inguinal glands were fixed in 4% paraformaldehyde overnight and subsequently embedded in paraffin wax, sectioned, and analyzed for human matrilysin expression by in situ hybridization and/or immunohistochemistry (data not shown).

Transgene expression at various ages of developement was detected by northern blot analysis in the mammary glands of transgenic females harboring the active, native, and inactive human matrilysin constructs (Figure 2). Transgene expression appeared to be most abundant in MMTV-ActMAT-22 (1 copy) and MMTV-NaMAT-3 (15 copies), and lower or absent in the remaining lines. Therefore we intend to expand these higher expressing lines for further evaluation. Additional glands are currently being processed to verify transgene expression in the MMTV-InMAT lines.

Whole mount preparations of the mammary glands from transgenic animals carrying the native human matrilysin construct revealed induction of alveolar structures in virgin transgenic mice when compared to glands from nontransgenic virgin animals (Figure 3). Ductal elongation and development of the primary branching structure appears to be the same in nontransgenic and transgenic animals, with the mammary epithelium growing to eventually fill the entire mammary fat pad. However, an

increased number of terminal alveoli have developed in the transgenic mammary glands. Histological evaluation of the inguinal mammary glands of the MMTV-NatMAT transgenic animals supports this observed morphological phenotype. Mammary glands from the transgenic animals carrying the active and inactive human matrilysin construct seem to have no apparent alterations in ductal branching structures (Figure 3).

FURTHER STUDIES:

Investigating the consequence of overexpressed human matrilysin protein in mammary tumorigenesis.

Several investigators have previously shown that matrilysin is overexpressed in human mammary cancers while only low levels, if any, have been detected in normal mammary tissue surrounding these tumors. Similarly, we have preliminary results indicating that metastatic murine mammary tumors also overexpress matrilysin mRNA, while normal mammary tissue expresses only very low levels of the matrilysin transcript. Therefore, we hypothesize that overexpression of matrilysin in induced mammary tumors may increase the aggressiveness of the tumors.

Administration of carcinogens such as 7,12-dimethylbenzantracene (DMBA) or N-nitrosomethylurea (NMU) have been shown to induce mammary tumors in rodents. However, carcinogen-induced rodent tumors frequently exhibit a well-differentiated morphology and low metastatic potential (1). In addition, approximately 75% of rodent mammary tumors induced by NMU, and 20% induced by DMBA exhibit a high incidence of altered or activated ras expression, which apparently occurs during initiation of these tumors (18). While alterations in ras expression can produce increase invasiveness and/or metastasis in ras-transfected human breast cancer cell line (19,20), altered or mutated ras expression occurs only infrequently in human breast cancer (21,22). In addition, the FVB strain of mice that we are utilizing for our transgenic studies tend to be naturally resistant to DMBA treatments (D. Medena and J. Rosen, personal communication).

neu/c-erbB2, on the other hand, has been observed to be amplified and overexpressed in a significant number of human breast cancers (23). Several studies have shown that the degree of amplification is inversely correlated to a poor clinical outcome (23,24). Because of this close correlation between neu overexpression and mammary carcinogenesis, transgenic mice have been generated that carry the native neu protein to directly test the oncogenic potential of the neu protein in mammary epithelium. Overexpression of the neu product in the mammary epithelium resulted in the appearance of focal mammary tumors in multiparous females by approximately 205 days that metastasized to the lungs in 72% of tumor bearing animals (25). Histological examination of the tumors revealed focal mammary adenocarcinomas surrounded by hyperplastic mammary epithelium. Transplantation of the

tumor cells into syngeneic recipients resulted in the appearance of tumors which confirms their neoplastic potential (25).

We have chosen to induce mammary tumors by crossing our MMTV-matrilysin female transgenic animals with the MMTV-neu male transgenic animals. We are currently breeding the two transgenic lines and will continue to do so until 25 female animals per group (neu alone, matrilysin alone, and neu/matrilysin crosses) are raised, giving a total of 75 female animals. Tumors are normally expected in half of the multiparous females harboring the neu transgene by approximately 205 days (25). We hypothesize that alterations in mammary tumorgenicity may be altered in the double transgenics due to the presence of the matrilysin protein.

Onset of tumors will be determined by palpitation of the mammary tissue. When progression of tumors hinder the animals movement and overall state of health, the animal will be euthanized and tumor and adjacent lymph nodes removed. The lungs and other organs will be removed and examined histologically for evidence of metastasis. A fraction of each tumor will be embedded and examined histologically for evidence of invasion into adjacent normal tissue, as well as analyzed by in situ hybridization for the localization of the matrilysin transgene. The remaining portion of each tumor will be snap frozen and later analyzed by northern analysis for the presence of human matrilysin gene expression.

CONCLUSIONS:

The long term goal of this proposal is to understand the molecular mechanisms that regulate matrilysin expression during both normal and neoplastic growth. We hope to determine the mechanisms by which matrilysin functions during mammary carcinogenesis by utilizing the mammary gland as an in vivo model system,

We have successfully generated transgenic animals that express native, active, and inactive human matrilysin in the mammary epithelium. Furthermore, we have preliminary data indicating that mammary development has been accelerated as a consequence of this transgene. Studies to address whether mammary carcinogenesis can be modified by overexpression of matrilysin are currently in progress.

ABSTRACTS:

A poster was presented at the Mammary Gland Biology Gordon Conference (June 18-23, 1995) titled:

> Alterations resulting from the overexpression of the matrix metalloproteinase matrilysin in the murine mammary gland.
> Laura A. Rudolph and Lynn M. Matrisian

REFERENCES:

1. Witty, J. P., J. Wright, and L. M. Matrisian. 1994. Matrix metalloproteinases are expressed during ductal and alveolar mammary morphogenesis, and misregualtion of stromelysin-1 in transgenic mice induces unscheduled alveolar development. *Mol. Biol. Cell.* In press (October issue).

2. Talhouk, R. S., J. R. Chin, E. N. Unemori, Z. Werb, and M. J. Bissell. 1991. Proteinases of the mammary gland: developmental regulation in vivo and vectorial secretion in culture. *Development* 112:439-449.

3. Wilson, C. L., K. J. Heppner, L. A. Rudolph, and L. M. Matrisian. 1995. The metalloproteinase matrilysin is preferentially expressed in epithelial cells in a tissue-restricted pattern in the mouse. *Mol. Biol. Cell* 6:851-869.

4. Saarialho-Kere, U. K., E. C. Crouch, and W. C. Parks. 1995. The matrix metalloproteinase matrilysin is constitutively expressed in adult human exocrine epithelium. *J. Invest. Dermatology* In Press.

5. Wolf, C., N. Rouyer, Y. Lutz, C. Adida, M. Loriot, J.-P. Bellocq, P. Chambon, and P. Basset. 1993. Stromelysin 3 belongs to a subgroup of proteinases expressed in breast carcinoma fibroblastic cells and possibly implicated in tumor progression. *Proc. Natl. Acad. Sci. USA* 90:1843-1847.

6. Papastoitsis, G., R. Siman, R. Scott, and C. R. Abraham. 1994. Identification of a metalloprotease from Alzheimer's disease brain able to degrade the ß-amyloid precursor protein and generate amyloidogenic fragments. *Biochemistry* 33:192-199.

7. Muller, D., B. Quantin, M. Gesnel, R. Millon-Collard, J. Abecassis, and R. Breathnach. 1988. The collagenase gene family in humans consists of at least four members. *Biochem J* 253:187-192.

8. Pajouh, M., R. Nagle, R. Breathnach, J. Finch, M. Brawer, and G. Bowden. 1991. Expression of metalloproteinase genes in human prostate cancer. *J Cancer Res Clin Oncol* 117:144-150.

9. Miyazaki, K., Y. Hattori, F. Umenishi, H. Yasumitsu, and M. Umeda. 1990. Purification and characterization of extracellular matrix-degrading metalloproteinase, matrin (pump-1), secreted from human rectal carcinoma cell line. *Cancer Res.* 50:7758-7764.

10. Witty, J. P., S. McDonnell, K. Newell, P. Cannon, M. Navre, R. Tressler, and L. M. Matrisian. 1994. Modulation of matrilysin levels in colon carcinoma cell lines affects tumorigenicity in vivo. *Cancer Res.* 54:4805-4812.

11. Sympson, C. J., R. S. Talhouk, C. M. Alexander, J. R. Chin, S. M. Clift, M. J. Bissell, and Z. Werb. 1994. Targeted expression of stromelysin-1 in mammary gland provides evidence for a role of proteinases in branching morphogenesis and the requirement for an intact basement membrane for tissue-specific gene expression. *J. Cell Biol.* 125:681-693.

12. Witty,J. P., T. Lempka, R.J. Coffey ,Jr., L. M. Matrisian. 1995. Decreased tumor formation in 7.12-dimethylbenzanthracene-treated stromelysin-1 transgenic mice is associated with alterations in mammary epithelial cell apoptosis. *Cancer Res.* 55:1401-1406.

13. Matsui, Y., S. Halter, J. Holt, B. Hogan, and R. Coffey. 1990. Development of mammary hyperplasia in MMTV-TGF a transgenic mice. *Cell* 61:1147-1155.

14. Sanchez-Lopez, R., R. Nicholson, M.-C. Gesnel, L. M. Matrisian, and R. Breathnach. 1988. Structure-function relationships in the collagenase family member transin. *J. Biol. Chem.* 263:11892-11899.

15. Park, A. J., L. M. Matrisian, A. F. Kells, R. Pearson, Z. Yuan, and M. Navre. 1991. Mutational analysis of the transin (rat stromelysin) autoinhibitor region demonstrates a role for residues surrounding the "cysteine switch". *J. Biol. Chem.* 266:1584-1590.

16. Henrard, D. and S. R. Ross. 1988. Endogenous mouse mammary tumor virus is expressed in several organs in addition to the lactating mammary gland. *J. Virol.* 62:3046-3049.

17. Matsui, Y., S. A. Halter, J. T. Holt, B. L. M. Hogan, and R. J. Coffey. 1990. Development of mammary hyperplasia and neoplasia in MMTV-TGFa transgenic mice. *Cell* 61:1147-1155.

18. Cha, R. S., W. G. Thilly, and H. Zarbl. 1994.
N-Nitro-N-methylurea-induced rat mammary tumors arise from cells with preexisting oncogenic Hras1 gene mutations. *PNAS* 91:3749-3753.

19. Kyprianou, N. and J. T. Isaacs. 1990. Relationship between metastatic ability and H-ras oncogene expression in rat mammary cancer cells transfected with the v-H-ras oncogene. *Cancer Res* 50:1449-1454.

20. Ichikawa, T., N. Kyprianou, and J. T. Isaacs. 1990. Genetic instabilty and the acquisition of metastatic ability by rat mammary cancer cells following v-H-ras oncogene transfection. *Cancer Res* 50:6349-6357.

21. Rochlitz, C. F., G. K. Scott, J. M. Dodson, E. Liu, C. Dollbaum, H. Smith, and C. C. Benz. 1989. Incidence of activating ras oncogene mutations

associated with primary and metastatic human breast cancer. *Cancer Res* 49:357-360.

22. Garcia, I., P. Y. Dietrich, M. Aapro, G. Vauthier, L. Vadas, and E. Engel. 1989. Genetic alterations of c-myc, c-erbB-2, and c-Ha-ras protooncogenes and clinical association in human breast carcinomas. *Cancer Res* 49:6675-6679.

23. Slamon, D. J., G. M. Clark, S. G. Wong, W. J. Levin, A. Ullrich, and W. L. McGuire. 1987. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogne. *Science* 235:177-182.

24. Varley, J. M., J. E. Swallow, W. J. Brammar, J. L. Wittaker, and R. A. Walker. 1987. Alterations to either c-erbB-2(neu) or c-myc proto-oncogenes in breast carcinoma correlates with poor short-term prognosis. *Oncogene* 1:423-430.

25. Guy, C. T., M. A. Webster, M. Schaller, T. J. Parsons, R. D. Cardiff, and W. J. Muller. 1992. Expression of the neu protooncogene in the mammary epithelium of transgenic mice induces metastatic disease. *Proc. Natl. Acad. Sci. USA* 89:10578-10582.

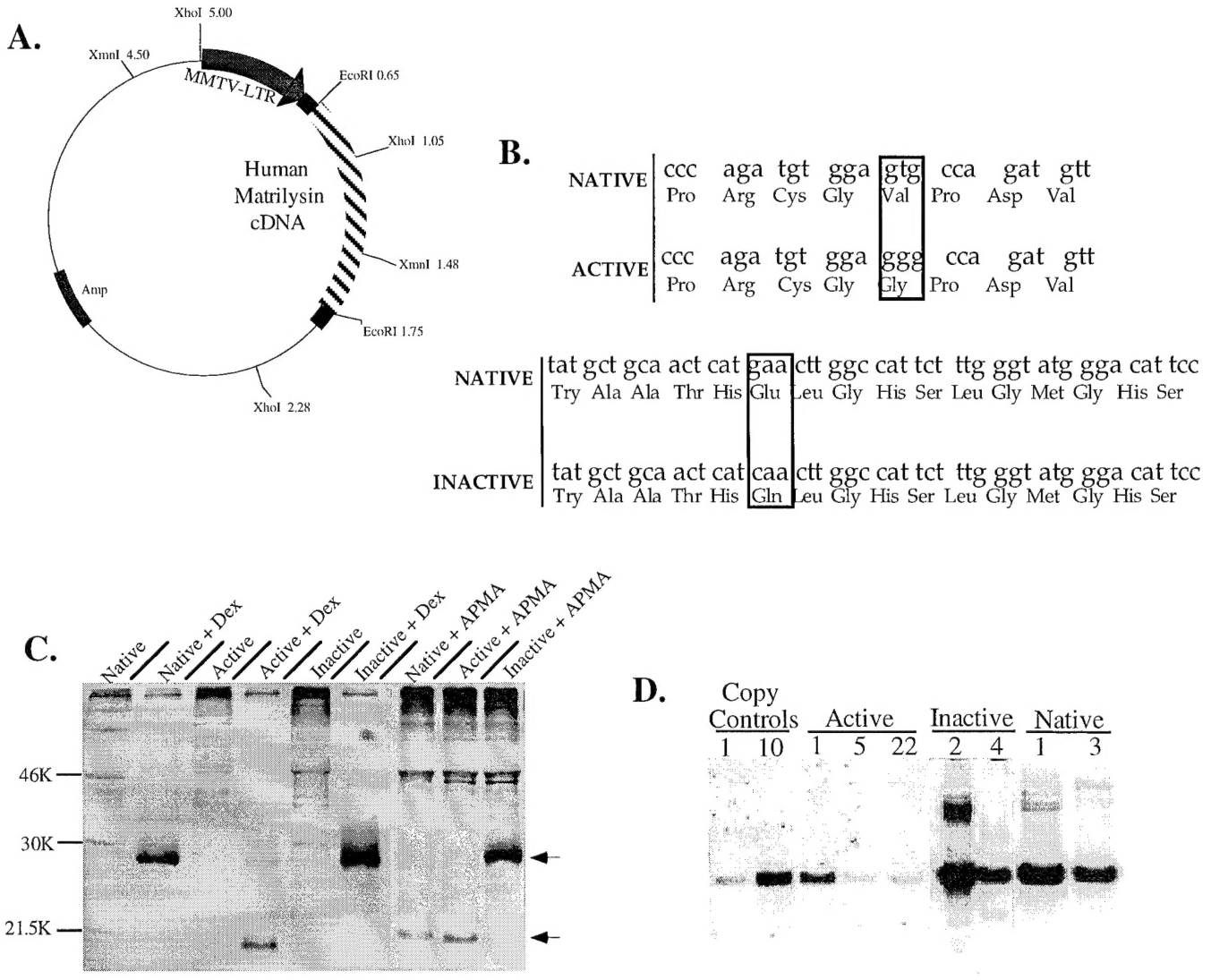

Figure 1: Generation of MMTV-matrilysin transgenic animals. A) Diagram of the plasmid construct utilized for creation of transgenics. Shaded region corresponds to the 1.5kb of the MMTV-LTR. The filled regions correspond to the rabbit b-globin gene. Three forms (inactive, active, and native) of the human matrilysin cDNA was inserted into the third exon of the b-globin gene. The open region corresponds to the expression vector pKCR sequence, with the gene for ampicillin resistance indicated. B) Native, active, and inactive matrilysin cDNA sequence and corresponding amino acids. Boxed areas indicate the position of the nucleotide mutation and amino acid substitution. C) Immunoprecipitation of the matrilysin protein. The breast cancer cell line Hs578t was transiently transfected with each MMTV-matrilysin construct, and the matrilysin protein immunoprecipitated from the conditioned media as indicated by the arrows. The active construct exhibits an increased tendency to undergo spontaneous conversion to the lower molecular weight mature form. Addition of 1μM of dexamethasone to the culture media had an inductive affect on all the MMTV-matrilysin constructs. Addition of APMA, a known MMP activator, to the condition media converted only the native and active forms to the lower molecular weight mature forms indicating that the inactive mutation prevents the enzyme from becoming cleaved and therefore fully activated. D) MMTV-matrilysin transgenic lines. Southern hybridization of 10μg of genomic DNA from founder animals, probed with a 1.1kb full length 32P-labelled human matrilysin cDNA probe.

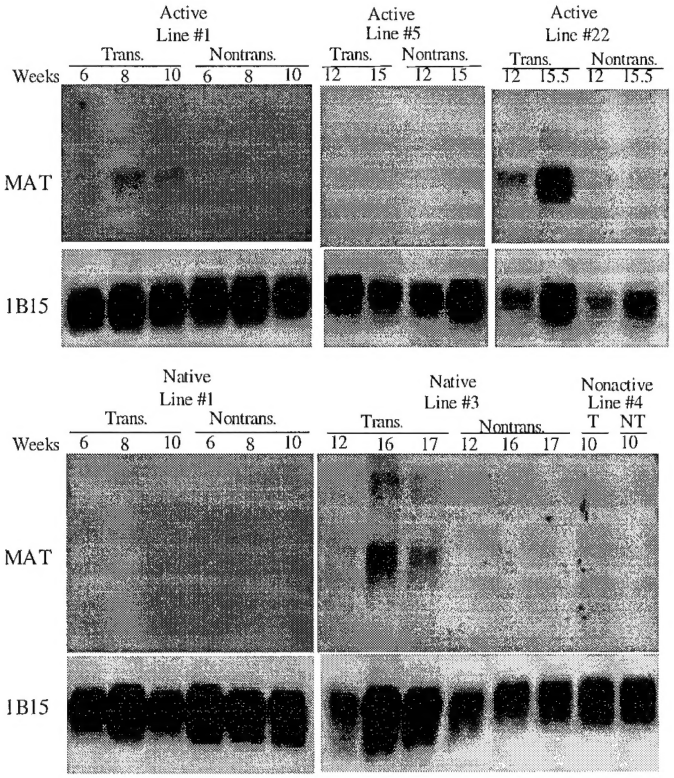

Figure 2: Expression of the human matrilysin transgene in developing mammary tissue. Northern analysis of poly A+ selected RNA (4μg) from nontransgenic and transgenic female mammary tissue at various weeks during mammary development. To identify the human matrilysin transgene (MAT), blots were probed with a 1.1kb full length 32P-labelled human matrilysin cDNA probe. Cyclophilin (1B15) cDNA probe was used to control for equal loading of the RNA.

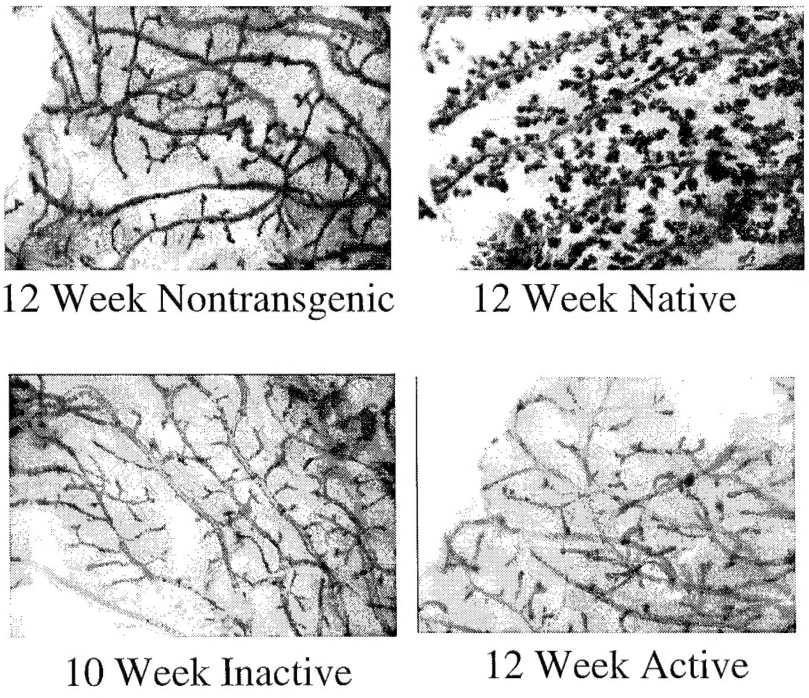

Figure 3: Morphological appearance of transgenic juvenile mammary glands. Whole mount preparations of mammary glands from 12 week native and active and 10 week inactive transgenics and littermate controls.